# Sex ratio of immature Kemp's ridley turtles
# (*Lepidochelys kempi*) from Gullivan Bay,
# Ten Thousand Islands, south-west Florida

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The sex ratio of 100 immature Kemp's ridley turtles (*Lepidochelys kempi*) captured in Gullivan Bay, Ten Thousand Islands, south-west Florida was determined through radioimmunoassay analysis (RIA) of plasma testosterone levels. We determined the sex ratio to be 1.9F:1.0M, corroborating the female dominance reported from other studies. The mean sizes of female (N=57) and male turtles (N=30) were 40.9 cm (±6.9 SD) and 41.9 cm (±6.2 SD), respectively. There were an additional 13 turtles (41.3 cm ±6.5 SD) for which sex could not be determined by this technique. Female-biased sex ratios are not uncommon in reptiles with temperature dependent sex determination. If fecundity is not male-limited, the occurrence of a female-biased sex ratio could enhance the recovery of the endangered Kemp's ridley turtle.

## INTRODUCTION

The Kemp's ridley turtle (*Lepidochelys kempi*) has experienced a dramatic decline in population size, although there are indications that the population is beginning to recover (Witzell, 2004). Sex ratios of threatened and endangered sea turtles are important reproductive parameters used by population ecologists and conservationists. These parameters are particularly vital for generating population models, developing conservation and management strategies, and for monitoring population recovery efforts.

Sea turtles have temperature-dependent sex determination (TSD) during egg incubation (Mrosovsky, 1994; Wibbels et al., 2000; Wibbels, 2003), which may produce sex ratios different from the 1:1 ratio normally expected (Mrosovsky, 1994; Shine, 1999; Freedberg & Wade, 2001). The majority of sea turtle sex ratio studies, including Kemp's ridleys, have indicated female biased sex ratios, which we would now expect with the Gullivan Bay ridleys if survival and dispersal are not sex specific. Determining sex ratios of immature sea turtles is particularly difficult because there are no obvious secondary sexual characteristics until maturity, and because it is difficult to obtain sufficient sample sizes of all size-classes from developmental habitats. There are currently three methods of estimating sex ratios of immature sea turtles: (1) examination of the gonads from necropsied stranded turtles; (2) laparoscopic examination of gonads from living turtles; and (3) predicting sexes by using serum testosterone levels from blood samples.

Due to the rareness of the Kemp's ridley, it is difficult to capture statistically significant numbers of specimens to accurately predict population sex ratios. Previous sex ratio studies have therefore been generated from stranded specimens in the Gulf of Mexico (Shaver, 1991; Stabenau et al., 1996; Cannon, 1998) and from the north-eastern United States (Morreale et al., 1992). Laparoscopic examination of juvenile turtles is extremely invasive and time consuming, and this procedure is therefore not commonly used to determine sex ratios of large numbers of young sea turtles. Researchers are also able to estimate sex ratios of immature turtles by determining testosterone levels in the blood by utilizing a radioimmunoassay (RIA) technique developed by Owens et al. (1978), and Coyne (2000) refined the technique for Kemp's ridleys by using laparoscopy to validate the results of the RIA procedure. Examining sex ratios of immature turtles from developmental habitats using RIA is relatively simple and allows large numbers of samples to be quickly collected. These large scale RIA studies of immature turtles can provide results that would avoid bias that may occur when sampling adults due to possible sex-specific migrations as occurs with loggerhead turtles (*Caretta caretta*) along the south-eastern coast of the United States (Henwood, 1987). Plasma testosterone levels have been used to determine sex ratios of immature Kemp's ridleys from the Texas-Louisiana border, north-western and west-central Florida developmental habitats (Coyne, 2000; Gregory & Schmid, 2001; Geis et al., in press). Results of these stranding and RIA studies vary, and may be influenced by sample size, geographical location, and size-class (Table 1). Unfortunately, the sample sizes of the two Florida studies are limited (N=36, Gregory & Schmid, 2001; N=42, Geis et al., in press) and it is felt that these studies required larger sample sizes to predict immature ridley sex ratios with confidence. In the current study, testosterone levels in the blood are used to predict the sex ratio of a large sample

**Table 1.** *Reported sex ratios of Kemp's ridley turtles. The sex ratios of stranded and alive turtles were determined by necropsy and radioimmunoassay, respectively.*

| Source | Location | Sex Ratio | N | Reference |
|---|---|---|---|---|
| Stranded | | | | |
| | Lower Texas | 1.0F:1.8M | 39 | Shaver (1991) |
| | Lower Texas | 3.2F:1.0M | 144 | Stabenau et al. (1996) |
| | Upper Texas | 0.8F:1.0M | 89 | Cannon (1998) |
| | New York | 2.0F:1.0M | 21 | Morreale et al. (1992) |
| Alive | | | | |
| | North-west Florida | 3.7F:1.0M | 42 | Geis et al. (in press) |
| | Female | | 33 | |
| | Male | | 9 | |
| | Intermediate | | 0 | |
| | Texas/Louisiana | 1.4F:1.0M | 239 | Coyne (2000) |
| | Female | | 134 | |
| | Male | | 95 | |
| | Intermediate | | 10 | |
| | West-central Florida | 1.8F:1.0M | 36 | Gregory & Schmid (2001) |
| | Female | | 23 | |
| | Male | | 13 | |
| | Intermediate | | 3 | |
| | South-west Florida | 1.9F:1.0M | 100 | Witzell et al. (this study) |
| | Female | | 57 | |
| | Male | | 30 | |
| | Intermediate | | 13 | |
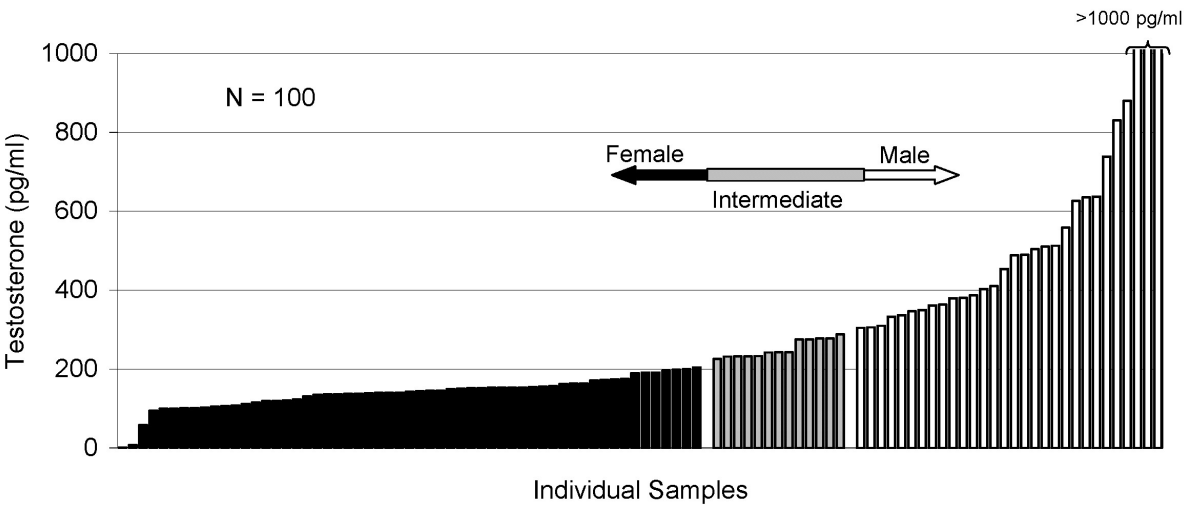


**Figure 1.** Testosterone levels for individual Kemp's ridley turtles from Gullivan Bay, Ten Thousand Islands, south-west Florida.

(N=100) of immature Kemp's ridley turtles from south-west Florida.

## MATERIALS AND METHODS

Kemp's ridley sea turtles were captured during a research project designed to determine species composition, relative abundance, demographics, and habitat preferences of immature sea turtles in Gullivan Bay in the Ten Thousand Islands area of south-west Florida (Witzell & Schmid, 2004). The turtles were observed from a boat when they surfaced to breathe and a net was rapidly deployed to capture the animal. Carapace length measurements were made with vernier calipers along the midline from the anterior nuchal notch to the posterior notch between the supracaudal scutes (i.e. minimum straight carapace length or MSCL). Blood samples were taken from the dorsal cervical sinus on turtles (during the 1999–2000 turtle survey seasons) with a 3.8-cm 21-gauge needle, needle holder, and heparinized vacutainer. The samples were taken immediately after capture (5–10 min) to reduce possible stress-induced variances in testosterone
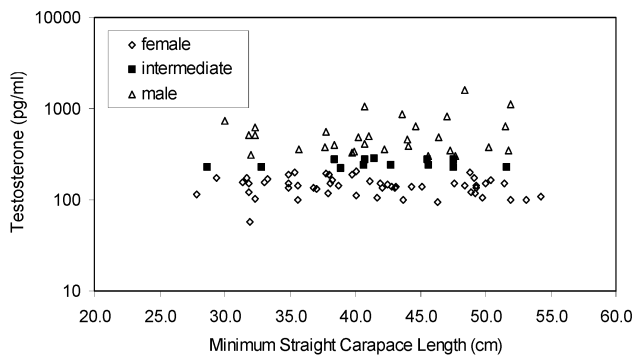
**Figure 2.** Carapace length (cm) and testosterone concentrations (pg/ml) for Kemp's ridley turtles from Gullivan Bay, Ten Thousand Islands, south-west Florida.

**Table 2.** *Mean sizes of wild female and male Kemp's ridley turtles.*

| Location | Sex | Mean Size (cm) | N | Reference |
|---|---|---|---|---|
| Texas/ Louisiana | | | | Coyne (2000) |
| | Female | 42.8 ±12.8 SD | 32 | |
| | Male | 37.3 ±2.3 SD | 26 | |
| West-central Florida | | | | Gregory & Schmid (2001) |
| | Female | 42.7 ±1.4 SD | 23 | |
| | Male | 42.3 ±1.7 SD | 13 | |
| South-west Florida | | | | Witzell et al. (this study) |
| | Female | 40.9 ±6.9 SD | 57 | |
| | Male | 41.9 ±6.2 SD | 30 | |

levels (Wibbels et al., 2000). The samples were immediately placed on ice and centrifuged upon arrival at a dockside facility, usually within 2–4 h after capture. The plasma was transferred into cryotubes and then stored in liquid nitrogen until the RIA analysis could be performed.

The RIA procedures used have been previously described by Owens et al. (1978), Wibbels et al. (1990), Coyne (2000), and Geis et al. (in press). Serial dilutions of testosterone standard and at least two control samples (nesting female and male pool) were also run to generate inter- and intra-assay coefficients. The testosterone RIA was validated for use with Kemp's ridleys using samples from immature animals of known sex from previous studies where sex had been verified by laparoscopy (Coyne, 2000; D.W. Owens, unpublished data). These data generated male and female ranges of testosterone levels that were used to assign sex to the turtles captured in the current study. The $G$-test for goodness of fit was used to evaluate significance of the predicted sex ratio from the expected 1:1 ratio.

## RESULTS AND DISCUSSION

Inter- and intra-assay coefficients of variation were 7.3% and 7.3% for the nesting female control A, and 4.7% and 5.4% for nesting female control B; 8.1% and 8.0% for the male pool control. The predicted female testosterone range for female and male turtles was 46.1 to 206.0 pg/ml, and 273.4 to 1078.0 pg/ml, respectively.

A total of 100 Kemp's ridley turtles were sampled during the 2000–2001 survey years. The testosterone levels and predicted sexes are shown in Figure 1. There were 13 turtles whose testosterone levels fell into the intermediate zone between the known male and female ranges. Therefore, the sexes of those 13 turtles were undetermined. The predicted sex of the remaining 87 turtles was: 57 female and 30 male for a ratio of 1.9F:1.0M. This ratio is significantly different from a 1:1 ratio ($G=8.52$, $P=0.004$). However, the 1.9F:1.0M ratio does not include the intermediate turtles. If these turtles were all female or all male, the ratio would be 2.3F:1.0M and 1.3F:1.0M, respectively.

The results indicate a female biased sex ratio (1.9:1.0) of immature Kemp's ridley turtles captured in south-west Florida. Sex ratios have been developed for Kemp's ridleys in other studies, all but one of which reported a female bias (Table 1). The predominance of female biases could be due to insufficient sample sizes, sex-specific survival and/or temporal dispersal, or errors in sexing methods. The south-west Florida ratio (1.9F:1.0M) is very similar to the ratio (1.8F:1.0M) from west-central Florida (Gregory & Schmid, 2001) but it is considerably lower than the ratio (3.6F:1.0M) for turtles from north-west Florida (Geis et al., in press), and it is higher than the ratio (1.4F:1.0M) from Texas (Coyne, 2000). There is no explanation for these differences, but inadequate sample sizes may have influenced either of the previous Florida studies. Also, the relative proportions of turtles in the intermediate category from these studies vary considerably. Perhaps the amount of indeterminate animals is temperature related, since the proportions of intermediate specimens increases from north (cold) to south (warm): north-west Florida (0.0%), Texas/Louisiana (4.2%), west-central (7.7%), and south-west Florida (13%).

The carapace lengths (MSCL) of predicted female, male, and intermediate turtles are presented (Figure 2) relative to testosterone level. The mean size of both sexes combined (N=87) was 41.4 cm MSCL. Mean female (N=57) and male (N=30) sizes were 40.8 cm MSCL (±6.9 SD) and 41.9 cm MSCL (±6.2 SD), respectively, and did not differ significantly ($z=-0.70$, $P=0.237$). The mean size of the intermediate turtles was similar (41.3 cm, ±6.5 SD). These mean sizes are similar to those lengths reported from other in-water studies (Table 2), and does not indicate any sexual dimorphism at this stage, assuming equal growth rates between sexes.

Female biased sex ratios have been estimated in other sea turtle species (see reviews by Mrosovsky, 1994; Wibbels, 2003), as well as in other reptiles with TSD. Such biases do not conform to the 1:1 predicted by Fisher (1930) for stable populations with both sexes having equal reproductive fitness. The occurrence of biased sex ratios in populations with TSD has led to a variety of evolutionary theories ranging from genetics to cultural inheritance (Shine, 1999; Freedberg & Wade, 2001). For example, Reinhold (1998) suggests that nest-site philopatry could provide a selective advantage for TSD in reptiles, causing the production of more females in high-quality nest sites and the production of more males in low-quality nest sites. Alternatively, the current findings could result from sex-specific dispersal patterns. Such a scenario has been

suggested for immature loggerhead turtles in the western Atlantic (Henwood, 1987). If this is the case, sex ratios could differ temporally and/or spatially.

The female biased sex ratio reported in the current study could be advantageous to the recovery of the Kemp's ridley turtle because female dominance could accelerate the recovery of a severely depleted ridley population by increasing egg production. And perhaps recovery is also enhanced by multiple paternity, where one male turtle might fertilize multiple nests with multiple partners. Kichler et al. (1999) found multiple paternity in Kemp's ridley turtles and concluded that the severe population crash did not adversely affect genetic diversity, suggesting that there were sufficient male turtles in the reproductive population. Whatever the reasons are for a female biased population, it is serendipitous because the population of Kemp's ridleys has apparently arrested its decline and is beginning to show signs of increase (Witzell, 2004).

## REFRENCES

Cannon, A.C., 1998. Gross necropsy results of sea turtles stranded on the upper Texas and western Louisiana coasts, 1 January–31 December 1994. In *Characteristics and causes of Texas Marine Strandings* (ed. R. Zimmerman). *United States Department of Commerce, National Oceanic and Atmospheric Administration (NOAA). Technical Report. National Marine Fisheries Service (NMFS)*, **143**, 81–85.

Coyne, M.S., 2000. *Population sex ratio of the Kemp's ridley sea turtle* (Lepidochelys kempii): *problems in population modeling*. PhD thesis, Texas A&M University, College Station, Texas, USA.

Fisher, R.A., 1930. *The genetical theory of natural selection*. Oxford: Clarendon Press.

Freedberg, S. & Wade, M.J., 2001. Cultural inheritance as a mechanism for population sex-ratio bias in reptiles. *Evolution*, **55**, 1049–1055.

Geis, A., Barichivich, W.J., Wibbels, T., Coyne, M., Landry, A. & Owens, D., in press. Predicted sex ratio of juvenile Kemp's ridley sea turtles (*Lepidochelys kempii*) inhabiting the waters near Steinhatchee, Florida. *Copeia*.

Gregory, L.F. & Schmid, J.R., 2001. Stress responses and sexing of wild Kemp's ridley sea turtles (*Lepidochelys kempii*) in the northwestern Gulf of Mexico. *General and Comparative Endocrinology*, **124**, 66–74.

Henwood, T.A., 1987. Movements and seasonal changes in loggerhead turtle *Caretta caretta* aggregations in the vicinity of Cape Canaveral, Florida (1978-84). *Biological Conservation*, **40**, 191–202.

Kichler, K., Holder, M.T., Davis, S.K., Marquez, R. & Owens, D.W., 1999. Detection of multiple paternity in the Kemp's ridley sea turtle with limited sampling. *Molecular Ecology*, **8**, 819–830.

Morreale, S.J., Meylan, A.B., Sadove, S.S. & Standora, E.A., 1992. Annual occurrence and winter mortality of marine turtles in New York waters. *Journal of Herpetology*, **26**, 301–308.

Mrosovsky, N., 1994. Sex ratios of sea turtles. *Journal of Experimental Zoology*, **270**, 16–27.

Owens, D.W., Hendrickson, J.R., Lance, V. & Callard, I.P., 1978. A technique for determining sex of immature *Chelonia mydas* using radioimmunoassay. *Herpetologica*, **34**, 270–273.

Reinhold, K., 1998. Nest-site philopatry and selection for environmental sex determination. *Evolutionary Ecology*, **12**, 245–250.

Shaver, D.J., 1991. Feeding ecology of wild and head-started Kemp's ridley sea turtles in South Texas waters. *Journal of Herpetology*, **25**, 327–334.

Shine, R., 1999. Why is sex determined by nest temperature in many reptiles? *Trends in Ecology and Evolution*, **14**, 186–189.

Stabenau, E.K., Stanley, K.S. & Landry, A.M., 1996. Sex ratios from stranded sea turtles on the upper Texas coast. *Journal of Herpetology*, **30**, 427–430.

Wibbels, T., 2003. Critical approaches to sex determination in sea turtle biology and conservation. In *Biology of sea turtles*, vol. 2 (ed. P. Lutz et al.), pp. 103–134. Boca Raton: CRC Press.

Wibbels, T., Owens, D.M. & Limpus, C.J., 2000. Sexing juvenile sea turtles: is there an accurate and practical method? *Chelonian Conservation and Biology*, **3**, 756–761.

Wibbels, T., Owens, D.W., Limpus, C.J., Reed, P.C. & Amoss, M.S., 1990. Seasonal changes in serum gonadal steroids associated with migration, mating, and nesting in the loggerhead sea turtle (*Caretta caretta*). *General and Comparative Endocrinology*, **79**, 154–164.

Witzell, W.N., 2004. Reproductive parameters of the Kemp's ridley sea turtle (*Lepidochelys kempii*) at Rancho Nuevo, Tamaulipas, Mexico: results of research conducted during the 1999–2000 nesting season. *Chelonian Biology and Conservation*, **4**, 779–785.

Witzell, W.N. & Schmid, J.R., 2004. Immature sea turtles in the Gullivan Bay, Ten Thousand Islands, and southwest Florida. *Gulf of Mexico Science*, **22**, 54–61.